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BIOCHEMICAL AND PHYSIOLOGICAL STUDY OF THE REST PERIOD IN THE TUBERS OF SOLANUM TUBEROSUM[1]

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(WITH TWO FIGURES)

Introduction

The cause and control of the rest period in plants have long been the subject of study, not only on account of their scientific interest, but also on account of the practical values which would accrue in many cases if the rest period were subject to control. Although many seeds have a rest period, this paper is concerned mainly with the rest periods in stems.

In spite of the immense amount of work that has been done on this problem, there still exist today two schools of thought, which stand directly opposed to each other on the first and most fundamental question involved, one school claiming that the rest period is a direct response to changing external conditions, while the other considers it to be the result of fixed, hereditary, internal causes. A middle ground is taken by others who attribute the rest period to a "cooperation of an hereditary tendency to rhythm, with the after effect of periodic repetition of reactions induced by external conditions." This diversity of opinion may be attributed largely to the variety of material used for the study of the problem and to the lack of exact and reliable experimental data.

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[1] Contribution from the Laboratory of Plant Physiology, Maryland Agricultural Experiment Station. Published by permission of the Director.

Historical

As examples of the extreme views in the older literature, we may mention those of GRISEBACH and ASKENASY. The former considered the yearly periodicity of plants of the temperate regions entirely a hereditary property, induced probably by physiological selection due to alternating external conditions through a long series of years; whereas the latter asserted that it is due directly to external conditions. SACHS believed that the rest period is caused by a deficiency of soluble food, and the cessation of the rest is due to a gradual production of enzymes. He does not state whether internal or external causes are responsible for the deficiency of food, nor does he suggest the nature of the process which increases the enzymes. FISHER (8) studied the carbohydrate transformations in woody stems during the rest period, and concluded that periodicity of growth is conditioned by a periodicity of the processes of food changes, which in turn rest upon a hereditary periodicity of certain properties of the protoplasm.

Coming now to the more modern literature, the studies of MÜLLER-THURGAU and SCHNEIDER-ORELLI (19–22) are of importance in the solution of this problem. They think the winter rest of plants is not conditioned by low temperature only, but the cessation of growth rests also upon the internal properties of the protoplasm of the meristem. These internal properties are inherited effects of winter cold on the growth process which after a long time become fixed in the protoplasm.

HOWARD (10) experimented with a large number of trees and shrubs, all deciduous species native to the temperate zone. He first tested their ability to grow in winter under favorable greenhouse conditions and then subjected to artificial treatments those which failed. Discussing the results of the foregoing experiments, the author states: "This shows conclusively that the majority of species, indigenous to the temperate climates, do not have firmly fixed winter rest periods from which they cannot be awakened." Although his observations were made on plants in winter rest, he includes also summer rest periods in his general conclusions. He thinks both forms of rest are caused by unfavorable external conditions. If these conditions, such as cold or drought, occur at regular intervals, a plant readily adapts itself to the new demands and the rest becomes a habit, which may continue to be repeated automatically for a longer or shorter period of time. The habit of rest thus induced is often very strongly fixed and is apparently transmissible.

Euler (7) attributes the rest period to internal self-regulatory processes and theorized in the following ingenious manner regarding the nature of these processes. The growth of a young cell shows two phases which follow each other by self-regulation. The first or stretching phase shows an increase of soluble osmotically active cell constituents; the second is characterized by the building up of the soluble materials into insoluble or colloidal molecules. Hydrolysis and synthesis, however, proceed simultaneously, and the two phases are characterized by an excess of the one process over the other. The synthesis of the second phase fixes the stretching of the first phase. What applies to the single cell applies also to the development of the entire organ and organism. In a number of cases, new cycles of stretching and condensation follow one another with unbroken regularity. In many other cases, a new cycle does not immediately follow upon the ending of a previous one. The stretching phase is prevented on account of an inability to dissolve the highly complex reserve materials. A short or long rest period must first take place. Finally a point is reached, and this point determines the length of the rest period, when the synthetic processes no longer hold the simultaneously occurring hydrolytic processes in equilibrium, and as a result internal osmotic pressures are increased. The resting organ is now physiologically ripe and begins to germinate, external conditions being favorable. As long as condensation processes predominate in the resting organ, germination is impossible. According to Euler, therefore, after-ripening would simply consist in certain changes during the rest period which weaken the synthetic processes. This theory, however, does not explain the origin and character of these changes.

The important observations of Schimper (25) gave us the first real knowledge of the peculiar behavior of tropical vegetation in respect to growth and rest periods. In places where moisture and temperature conditions are favorable during the entire year, many plants show a rhythmic alternation of periods of rest and activity of the growth processes. SCHIMPER states: "Internal causes are mainly or solely responsible for the alternation of rest and activity in a nearly uniform climate. Such a rhythmic change, however, is never abandoned, for it arises from the nature of the living organism and not from external conditions; its connection with external conditions is a secondary feature, an adaptation."

From the time of SCHIMPER'S observations, tropical vegetation has been favorite material for study in determining the relation of external conditions to rest periods. KLEBS (**16**) has made observations on the tropical vegetation of Java, where climatic conditions show little variation. Exact measurements of twigs were made to determine the amount of growth over a series of months. He also cultivated a number of European and Japanese plants in Java. Native plants of Java were likewise transplanted to Heidelberg and grown in a greenhouse under constant temperature and moisture conditions. These studies, which constitute his most recent research on this problem, led him to deny firmly a periodicity of growth which is independent of external factors, and to formulate the following hypothesis to account for this rest period in tropical trees: When the growth ability is weakened by a deficiency of one or more of the essential external growth factors, carbon assimilation proceeds at first at the normal rate, resulting in a storage of organic materials. These in turn render certain enzymes inactive and thus cause a rest period. All means available for shortening the rest period simply activate these enzymes. Great stress is laid upon salts as the causal agent in the rest periods of tropical plants, since the other three factors are constantly favorable for growth in Java.

VOLKENS (**28, 29**) made more extended observations on the tropical vegetation in its natural habitat. Trees were marked and observed for an entire year. Many new and interesting facts were added to our stock of knowledge regarding rest and activity in tropical plants. Although his observations were made in the same place as those of KLEBS and often on the same object, he reached a very different conclusion. He is unable to see any relation between the rest periods in tropical plants and external conditions;

they must be due to internal causes. He does not intimate, however, the nature of these causes.

It is true that VOLKENS does not include the factor of salts among the external conditions that may affect the growth processes; and besides, he and KLEBS do not seem to have quite the same conception of internal causes, so that their views may not be as antagonistic as first seems. According to VOLKENS, everything is conditioned by internal causes which cannot be brought into evident connection with external factors. The important thing to KLEBS is whether the rest is caused by the outer world or is an expression of an internal fixed "specific structure." VOLKENS does not discriminate between specific structure and internal conditions. It is conceivable that a slight external change may set into motion a chain of effects which would ultimately so change the internal conditions of the growing cell or its immediate environment that growth would be arrested and seem to be due to internal specific structure.

Attempts to shorten the rest period of buds and bulbs by artificial means are numerous and some have been successful. MÜLLER-THURGAU (20) claims to have shortened the rest period of potato tubers by one month's storage at 0° C. The use of ether and chloroform dates from the important work of JOHANNSEN (14), who succeeded by their means in forcing buds to open 3–6 weeks earlier than normally. He found, however, that these agents are effective only at the beginning and near the end of the rest periods. The warm bath has been used successfully in shortening the rest period. MOLISCH (18) caused an earlier opening of buds by immersing the shoots in water at 35° C. MÜLLER-THORGAU and SCHNEIDER-ORELLI (22) hastened the growth of lily-of-the-valley bulbs and potato tubers by warming them at a temperature of 38° C. and maintaining a germination temperature of 26° C. Warm air was equally as effective as warm water. In an endeavor to test KLEBS's conception regarding the importance of salts in bud growth, LAKON (17) stood cut twigs of trees and shrubs in Knop's solution and found that of a large number only one failed to show an earlier unfolding of the buds than normally. This does not prove, however, that the buds failed to germinate on account of the lack of salts any more than the lack of ether prevented germination in

JOHANNSEN's experiments. JESENKO (12) found that hydrochloric, sulphuric, and tartaric acids, as well as alcohol and water saturated with carbon dioxide, hastened the germination of buds of a number of woody plants. He concluded that the applied solutions not only acted as a stimulus in the strict sense of the word, but also started certain chemical processes in the buds which produced favorable conditions for growth. JOST (13) observed that wounded potatoes, especially if they were cut into many pieces, showed an earlier germination of the buds than unwounded ones.

WISMEWSKI (31) experimented with the winter buds of certain aquatic plants. The rest period of the buds of *Hydrocharis Morsus-ranae* could be shortened by wounding and lengthened by darkness. *Hydrocharis* formed buds the entire winter when kept in darkness and immersed in rainwater or a nutritive solution. He concludes that neither the origin nor germination of these buds is conditioned by an inner rhythm of the plants. He also states that low temperature is not a necessary condition for the origin of buds.

Although the rest period may be shortened by artificial treatments, none has thus far been capable of entirely eliminating the rest period where it is well fixed. A great deal of work has been done on forcing resting stem structures into growth by artificial means, but comparatively little has been done to determine the character of the physiological and chemical changes effected by such treatments. Still less is known about these changes during the natural rest period.

MÜLLER-THURGAU and SCHNEIDER-ORELLI (21, 22) found increased respiration in potato tubers and lily-of-the-valley bulbs after the warm-bath treatment and also after etherizing.

IRAKLIONOW (27) also found a rise in respiration of potato tubers after treatment with warm water. At the end of a few days, however, it fell back to the normal rate and did not rise again until the beginning of germination. He assumes, in agreement with MOLISCH, that the breaking of the rest period by means of the warm bath is a simultaneous action of the high temperature and the water, and that the warm bath influences the enzymes, chiefly the oxidases.

Butkewitsch (4) showed that the stored starch in the cortex and wood can be dissolved by the action of toluol and chloroform, and also by high temperature. He thinks the action is similar to cold and consists in a weakening of the plastids.

Grüss (9) investigated the chemical changes set up in the cells around the wound in potatoes, and found an increase in the oxidizing enzymes and in the diastase activity. There was some sugar accumulation in the subphellogen. Müller-Thurgau (19) was probably the first to observe the accumulation of sugar in potatoes stored at low temperatures.

Fisher (8) studied the changes in the starch content of trees during the rest period. He found the maximum of starch from leaf fall to the beginning of November. Starch solution began in November and reduced the starch content to the minimum in the winter. Starch synthesis began in March and reached the maximum again in April, after which hydrolysis began and brought the starch content to the minimum during the latter part of May, or the time of the beginning of vegetative activity. Storage of starch occurred of course during the summer.

Biochemical

It is obvious from the foregoing survey of the literature that there is little exact experimental data on the chemical and physical situation in stems which forces the growing cells into a period of rest. The same is true of the changes in the resting tissue or its immediate environment which are essential to the release of the growth processes at the end of the rest period. These latter changes will be spoken of as after-ripening, using the term in its broadest sense.

It is a well known fact that under normal conditions potato tubers will not sprout for several weeks after harvest. Rehobeth potatoes harvested on July 17 and planted immediately in the same field did not sprout until October, although good growing conditions prevailed during the entire period. McCormick potatoes harvested on November 11, and kept constantly under favorable growing conditions in the greenhouse, did not sprout until January 24; general sprouting did not occur until February. It has been generally conjectured that the after-ripening processes in tubers are metabolic in character; in other words, it has been supposed that chemical changes occur in the tubers during the rest period which render certain essential foods or other materials available for the growing bud. The first problem in the following study was to ascertain if there are metabolic processes occurring in the potato tuber during the rest period which are characteristic of after-ripening, and to determine their character as far as possible.

The tubers used in this investigation produce sprouts much earlier from the buds on the seed or terminal end when the tubers are left whole. In the majority of cases, the buds on the stem or basal end do not germinate at all unless those on the seed end are injured; therefore, tubers were cut in half at regular intervals during the rest period, and the following analyses made separately on the seed and stem halves with a view to detecting better the chemical changes characteristic of after-ripening. We are not justified in assuming that all chemical changes occuring in the whole tuber during the rest period must be peculiar to after-ripening.

CARBOHYDRATES.—In a previous chapter it was stated that storage at low temperature has been claimed to be capable of shortening the rest period of potato tubers. It has been proved that during such storage sugar accumulates in the tubers. These facts have led to the supposition that the rest period is caused by a deficiency of soluble carbohydrates, and that the cessation of the rest is due to a gradual production of the diastase. In order to determine if carbohydrate transformations are essential after-ripening processes, or simply due to changing temperature, analyses were made during after-ripening of tubers at a fairly constant and favorable growing temperature. At the same time analyses were made on tubers stored under variable low temperature in a potato vault. Samples of 8 tubers, each with a total weight of about one kilogram, were selected from both lots at intervals of 2 and 4 weeks. Each tuber was cut into two equal parts, the one part representing the terminal or seed end, the other, the basal or stem end. The seed ends of each sample were all grated together; likewise the stem ends. After being thoroughly mixed in a mortar, both lots of pulp were sampled for determinations of moisture, starch, total sugar, reducing sugar, and diastase.[2]

The final carbohydrate results are calculated to original moisture basis. It is thus possible to record results in percentage of wet weight, as the possibility of apparent changes due to loss of water by evaporation is excluded. Apparent changes due to this water loss would also be excluded by calculating results to dry basis at the time of analysis, but these results might still show changes due to water taken up by hydrolysis, or to the accumulation of respiratory water, especially where evaporation is prevented. This method, however, does not take into account loss in dry matter through respiration. In the case of the vault-stored potatoes this would be negligible, since respiration is very low at the vault temperature.

Failure of growth in the buds during the rest period is not due to a lack of available sugar, since the percentage of both reducing and total sugars in the greenhouse-stored tubers was no greater when sprouting began than at the beginning of the rest period (tables I–IV); besides, the seed end at the time of sprouting did not contain a greater percentage of sugar than the stem end. *The carbohydrate transformations during the rest period are entirely dependent upon changing temperature, and must, therefore, not be considered after-ripening processes.*

DIASTASE.—Since the supply of soluble carbohydrates for the growing buds is dependent upon the important enzyme diastase, a gravimetric method was employed to determine the diastatic power of the juice from the two ends at intervals during after-ripening under greenhouse conditions. The determinations were made on the same samples used for the carbohydrate analyses.

The increase in total sugar after incubation was considered an index of the diastatic activity of the potato extract at the time of analysis. It may indicate simply the excess of the hydrolytic process over a simultaneously occurring synthetic process. On either basis, the potato extract contained active diastase at all times during the rest period. It was uniformly greater in the

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[2] For detailed descriptions of methods, as well as additional tables and figures, see Bull. no. 183. Maryland Agric. Exper. Sta.

extract from the seed end, but there was no appreciable increase in the extract from either end during the rest period. It may be concluded, therefore, that the *cessation of the rest period is not due to a gradual increase of diastase activity.*

TABLE I

REDUCING SUGAR; McCORMICK POTATOES PLANTED IN WET SAWDUST IN GREENHOUSE; TUBERS SPROUTING ON MARCH 26

DATE OF ANALYSIS	RANGE OF TEMPERATURE BETWEEN ANALYSES	REDUCING SUGAR CALCULATED ON BASIS OF ORIGINAL MOISTURE		
		Seed end per cent	Stem end per cent	Whole tuber per cent
November 14.......	....	0.287	0.320	0.304
December 14.......	20° C. to 22° C.	0.372	0.342	0.357
January 20.........	22° C. to 17° C.	0.478	0.481	0.479
February 3.........	20° C. to 22° C.	0.274	0.311	0.293
March 26..........	21° C. to 23° C.	0.385	0.401	0.393

TABLE II

REDUCING SUGAR; GREEN MOUNTAIN POTATOES STORED IN VAULT

DATE OF ANALYSIS	RANGE OF TEMPERATURE BETWEEN ANALYSES	REDUCING SUGAR CALCULATED ON BASIS OF ORIGINAL MOISTURE		
		Seed end per cent	Stem end per cent	Whole tuber per cent
November 28....	...... to 5° C.	0.655	0.745	0.70
December 20....	5° C. to 2° C.	0.972	1.40	1.19
January 13......	2° C. to −1.5° C.	1.35	1.85	1.60
February 13.....	−1.5° C. to − 1° C.	2.20	2.62	2.40

TABLE III

TOTAL SUGAR; McCORMICK POTATOES PLANTED IN WET SAWDUST IN GREENHOUSE; TUBERS SPROUTING ON MARCH 26

DATE OF ANALYSIS	RANGE OF TEMPERATURE BETWEEN ANALYSES	TOTAL SUGAR CALCULATED ON BASIS OF ORIGINAL MOISTURE		
		Seed end per cent	Stem end per cent	Whole tuber per cent
November 14.......	....	0.663	0.667	0.665
December 14.......	20° C. to 22° C.	0.572	0.503	0.538
January 20.........	22° C. to 17° C.	0.663	0.626	0.645
February 3.........	20° C. to 23° C.	0.430	0.413	0.408
March 26..........	21° C. to 23° C.	0.537	0.553	0.545

TABLE IV

Total sugar; Green Mountain potatoes stored in vault

Date of analysis	Range of temperature between analyses	Total sugar calculated on basis of original moisture		
		Seed end per cent	Stem end per cent	Whole tuber per cent
November 28....	.....to 5° C.	1.02	1.19	1.10
December 20	5° C. to 2° C.	1.48	1.76	1.62
January 13......	2° C. to −1.5° C.	1.94	2.26	2.10
February 13.....	−1.5° C. to − 1° C.	3.84	4.04	3.94

Oxidase.—The juice from new potatoes shows less ability to accelerate the oxidation of pyrogallol than the juice from tubers at the end of the rest period. The juice from the seed half causes no greater acceleration of this oxidation than that from the stem half, even after sprouting (tables VI–VIII).

TABLE V

Action of glycerine extract of potato pulp on soluble starch solution; McCormick potatoes stored in wet sawdust in the greenhouse

Date of analysis	Range of temperature between analyses	Increase in milligrams of sugar in 24 hours at 40° per 100 gm. of potato pulp					
		Reducing sugars			Total sugar		
		Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber
November 14 ..	....	65.0	58.0	61.5	65.6	60.0	62.8
December 14...	20° C. to 22° C.	63.2	56.0	59.5	86.3	72.0	79.0
January 20.....	22° C. to 17° C.	91.0	67.2	79.1	72.1	60.5	66.3
February 3.....	20° C. to 22° C.	84.0	66.4	76.8	78.4	63.9	71.1

Nitrogen.—A general survey of the different combinations of nitrogen in the tuber was made at intervals during the rest period in order to determine if protein hydrolysis or other transformations of the nitrogen-containing substances occur during the rest period as necessary antecedents to sprouting. The following determinations were made: total nitrogen, water-soluble nitrogen, nitrogen coagulated by heat, nitrogen precipitated by tannic and by phosphotungstic acids. It was assumed that the nitrogen coagulated by heat represented the protein nitrogen, and the difference between

TABLE VI

OXIDATION OF PYROGALLOL BY JUICE FROM IMMATURE TUBERS JUST HARVESTED AND BY JUICE FROM TUBERS OF THE SAME VARIETY AT THE END OF THE REST PERIOD, BUT NOT SPROUTING

ELAPSED TIME	TEMPERATURE AT THE TIME OF READING	MANOMETER READINGS EXPRESSED IN CENTIMETERS OF MERCURY	
		New tuber	Old tuber
Hours			
3	33°8 C.	−0.65	−0.7
22	33.8	−1.55	−1.9
25	34.0	−1.70	−2.2

TABLE VII

OXIDATION OF PYROGALLOL BY JUICE FROM NEW TUBERS AND FROM TUBERS OF THE SAME VARIETY AT THE END OF THE REST PERIOD, BUT NOT SPROUTING

ELAPSED TIME	TEMPERATURE AT THE TIME OF READING	MANOMETER READINGS EXPRESSED IN CENTIMETERS OF MERCURY		
		New tuber	Old tuber	Old tuber calculated to moisture of new tuber
Hours				
5	34°6 C.	−0.4	−0.6	−0.55
26	34.6	−0.8	−1.15	−1.05
46	34.6	−0.85	−1.35	−1.24
71	34.6	−1.50	−2.00	−1.83
125	34.6	−3.05	−3.4	−3.02

TABLE VIII

OXIDATION OF PYROGALLOL BY JUICE FROM THE SEED AND STEM HALVES, JANUARY 10

ELAPSED TIME	TEMPERATURE AT THE TIME OF READING	MANOMETER READINGS EXPRESSED IN CENTIMETERS OF MERCURY	
		Seed half	Stem half
Hours			
4	34°6 C.	−0.75	−0.75
6	34.7	−1.45	−1.45
48	34.5	−2.85	−2.90

this and the nitrogen precipitated by tannic acid, the proteose, and the peptone nitrogen. The nitrogen not precipitated by phosphotungstic acid was considered the nitrogen of monoamino acids and their amide derivatives, while the difference between this and the nitrogen not precipitated by the tannic acid was considered the nitrogen of diamino acids and other bases. It is not claimed that the foregoing precipitation method gives absolutely the true proportion of nitrogen in the various forms of binding, but it yielded valuable comparative results under the conditions employed in the determinations.

The nitrogen determinations were made on Green Mountain potatoes harvested on November 4 and planted at once in wet sawdust on the floor of the greenhouse. The temperature variation in the sawdust was slight and the tubers were constantly under favorable growing conditions. Sprouts began to appear on this lot of potatoes on January 19. The set of analyses made on January 18, therefore, shows the nitrogen situation just at the end of the rest period. The tubers used for the last set of analyses bore sprouts from one-eighth to one inch in length. As soon as sprouting began, the tubers were placed in a moist chamber, which was buried in the sawdust; therefore nothing was absorbed by the roots except possibly a little water. Each sample contained 6 tubers with a total weight of about 800 gm. The variation in total weights was not more than 5 gm. The samples were all weighed the day after the potatoes were harvested, and kept separate during the storage in the wet sawdust. This makes possible the calculation of results to percentage of original weight, and thus apparent changes due to loss in dry substance through respiration and also through changes in water content are avoided.

On the above basis of calculation the different forms of nitrogen in the whole tuber showed no general change until the tubers began to sprout. During the rest period the stem half always showed a higher percentage of nitrogen, calculated to percentage of total nitrogen, in the following forms: monoamino acids and amides, diamino acids and other bases, proteoses, and peptone. The seed half contained a slightly higher percentage of both water-soluble and water-insoluble protein nitrogen (tables IX–XI). *The slight* *variation in the relative magnitudes of the above forms of nitrogen during the rest period was no greater than would be expected in different biological samples; in most cases it was well within the experimental error.*

TABLE IX

NITROGEN OF THE WHOLE TUBER CALCULATED TO PERCENTAGE OF TOTAL WEIGHT OF EACH SAMPLE ON NOVEMBER 8; TUBERS SPROUTING ON FEBRUARY 12

Date of sampling	Total nitrogen	Water-soluble nitrogen	Nitrogen not coagulated by heat	Nitrogen not precipitated by tannic acid	Nitrogen not precipitated by phosphotungstic acid
November 10...	0.430	0.365	0.232	0.217	0.205
November 29...	0.432	0.371	0.234	0.222	0.210
December 13...	0.429	0.369	0.225	0.211	0.198
December 27...	0.428	0.367	0.234	0.222	0.213
January 18.....	0.424	0.345	0.229	0.217	0.205
February 12....	0.428	0.381	0.239	0.228	0.223

TABLE X

DISTRIBUTION OF NITROGEN IN PERCENTAGE OF TOTAL NITROGEN; TUBERS SPROUTING ON FEBRUARY 12

DATE OF ANALYSIS	WATER-SOLUBLE PROTEIN NITROGEN			WATER-INSOLUBLE PROTEIM NITROGEN			NON-PROTEIN NITROGEN		
	Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber
November 10.....	31.95	29.98	30.96	15.04	14.98	15.01	53.01	55.04	54.02
November 29.....	32.41	30.77	31.59	14.49	13.75	14.12	53.10	55.48	54.29
December 13.....	32.78	31.68	32.23	15.01	13.71	14.36	50.12	54.61	52.36
December 29.....	30.74	31.18	30.96	15.58	12.87	14.22	53.68	55.95	54.31
January 18.......	32.83	28.40	30.61	14.02	13.82	13.92	53.15	57.78	55.46
February 12......	32.82	30.62	32.81	11.96	10.68	11.30	55.24	58.70	56.97

TABLE XI

DISTRIBUTION OF NITROGEN IN PERCENTAGE OF TOTAL NITROGEN; TUBERS SPROUTING ON FEBRUARY 12

DATE OF ANALYSIS	PROTEOSE AND NEPTONE NITROGEN			NITROGEN OF DIAMINO ACIDS AND OTHER BASES			NITROGEN OF MONOAMINO ACIDS AND AMIDES		
	Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber
November 10.....	2.54	3.51	3.02	2.33	3.28	2.80	48.14	48.25	48.19
November 29.....	2.49	3.50	3.00	2.32	2.79	2.56	48.29	49.19	48.72
December 13.....	2.99	3.31	3.15	2.11	3.40	2.75	47.11	47.90	47.51
December 29.....	......	......	......	2.08	3.10	2.59	48.42	50.71	49.56
January 18.......	2.10	3.46	2.78	2.04	3.95	2.99	49.01	50.37	49.69
February 12......	3.71	3.69	3.70	1.11	1.80	1.45	50.43	53.21	51.75

PHOSPHORUS.—Phosphorus is an essential element of two of the most important constituents of the cell, the nucleoproteins and the lipoids. Protein, lipoid, and extractive phosphorus were determined at intervals during the rest period with a view to ascertaining if certain gradual transformations of the phosphorus combinations occur during the rest period in order to render phosphorus available in the proper form for the growing buds.

TABLE XII

EXTRACTIVE; PROTEIN AND LIPOID PHOSPHORUS CALCULATED TO PERCENTAGE OF TOTAL PHOSPHORUS; MCCORMICK POTATOES PLANTED IN WET SAWDUST IN THE GREENHOUSE; TUBERS SPROUTING ON MARCH 26

DATE OF SAMPLING	EXTRACTIVE P			PROTEIN P			LIPOID P		
	Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber	Seed end	Stem end	Whole tuber
November 14.....	49.1	47.57	48.33	44.34	45.90	45.12	6.65	6.54	6.58
December 14.....	50.50	46.76	48.63	43.50	47.13	45.31	6.003	6.099	6.006
January 20.......	50.20	44.90	47.55	42.88	48.36	45.62	6.915	6.763	6.840
February 3......	50.81	44.60	47.70	41.14	48.11	44.62	8.056	7.29	7.673
March 26........	52.83	50.08	51.45	37.99	41.99	39.99	9.198	7.93	8.514

TABLE XIII

INORGANIC PHOSPHORUS IN PERCENTAGE OF TOTAL PHOSPHORUS; GREEN MOUNTAIN POTATOES STORED IN WET SAWDUST IN THE GREENHOUSE; TUBERS SPROUTING ON FEBRUARY 12

DATE OF ANALYSIS	INORGANIC PHOSPHORUS		
	Seed end	Stem end	Whole tuber
November 29.............	34.23	33.90	34.06
December 13..............	35.75	34.59	35.17
December 27..............	33.22	33.08	33.15
January 18...............	32.71	34.11	33.41
February 12..............	32.00	34.30	33.15

Calculated to percentage of total phsophorus, it was found that the percentage of extractive phsophorus was consistently higher in the seed end than in the stem end; the percentage of protein phosphorus, on the other hand, was always less in the seed end. *The percentages of all the phosphorus combinations were practically constant throughout the rest period* (tables XII and XIII).

Physiological

Immature potatoes have a thin, slightly suberized skin, which is quite permeable to both water and gases. As the tubers mature, the skin becomes more suberized and more adherent to the underlying tissue. The rapidity and degree of suberization, however, is greatly influenced by moisture; dry conditions favor the process, while moisture retards it. As the skin becomes suberized, its permeability to water and gases is greatly reduced. It occurred to the writer that the skin may very soon become a sufficient barrier between the internal tissues and the external agents to check growth in the buds. This might be due to an external agent becoming a limiting factor in the completion of the growth mechanism in the new tuber, or in the growth itself. The following experiments were planned to test this hypothesis:

The fall crop of McCormick potatoes furnished material for the experiments here recorded, except where otherwise noted. All the experiments, however, were repeatedly confirmed with the summer crop of both Green Mountain and Rehobeth potatoes. The regular mature crop of the McCormick potatoes was harvested on November 4. Tubers were at once planted in the greenhouse in soil, sawdust and sphagnum, but in no case did sprouting occur until January 18; general sprouting did not begin until February. These results were confirmed by similar plantings of McCormick potatoes in 3 successive years. The rest period of McCormick potatoes under natural planting conditions, therefore, is about 90 days from the time the mature crop is harvested at this station. If they are harvested earlier, the rest period is much longer. Immature tubers harvested on September 20, and immediately planted in the greenhouse, did not sprout until February 2.

EFFECT OF REMOVING THE SKIN.—Simply removing the skin from potato tubers at any stage of the rest period will bring about sprouting within 10 days, if favorable external conditions prevail. A number of methods were employed in order to supply the most favorable conditions for sprouting; but the best among those tried consisted in planting the stem ends in wet soil or sawdust and covering the seed ends with 2 or 3 inches of excelsior, kept constantly wet. This method exposes the terminal buds to the maximum partial oxygen pressure of the atmosphere. The mere greening of the tubers in the subdued light affords considerable protection against decay. Under the foregoing condition the degree of previous corking of the skin greatly influenced the time before the tubers with the skin intact began to sprout.

Tables XIV–XVI record typical experiments which show the facts given above. The temperature of each pot was recorded

TABLE XIV

EFFECT OF REMOVING THE SKIN; IMMATURE TUBERS HARVESTED ON SEPTEMBER 20 AND PLANTED THE SAME DAY; 12 TUBERS IN EACH LOT

STEM ENDS PLANTED IN	SEED ENDS COVERED WITH	SKINS	PERCENTAGE SPROUTED AFTER						AVERAGE LENGTH OF SPROUTS AFTER 35 DAYS
			10 days	20 days	35 days	85 days	110 days	135 days	
Soil......	Soil	On	0	0	0	0	23	70	0
Soil......	Excelsior	On	54	62	77	77	......	85	4 mm.
Soil......	Excelsior	Off	38	100	100	100	100	100	20 mm.
Sawdust..	Sawdust	On	0	0	0	0	0	42	0
Sawdust..	Sawdust	Off	75	75	100	100	100	100	5 mm.
Sawdust..	Sawdust	Off	57	85	100	100	100	100	10 mm.

TABLE XV

EFFECT OF REMOVING THE SKIN; MATURE TUBERS HARVESTED ON OCTOBER 28 AND PLANTED ON OCTOBER 31; MEDIUM CORKED SKINS; 12 TUBERS IN EACH LOT

STEM ENDS PLANTED IN	SEED ENDS COVERED WITH	SKINS	PERCENTAGE SPROUTED AFTER			
			10 days	20 days	35 days	95 days
Soil........	Soil	On	0	0	0	16
Soil........	Excelsior	On	0	0	20	100
Soil........	Soil	Removed	0	0	25	82
Soil........	Excelsior	Removed	25	83	100	100

TABLE XVI

EFFECT OF REMOVING THE SKIN; MATURE TUBERS HARVESTED ON NOVEMBER 4 AND PLANTED ON NOVEMBER 22; HEAVILY CORKED SKINS

STEM ENDS PLANTED IN	SEED ENDS COVERED WITH	SKINS	PERCENTAGE SPROUTED AFTER			
			10 days	20 days	45 days	72 days
Sawdust.....	Excelsior	On	0	0	0	43
Sawdust.....	Excelsior	Removed	50	100	100	100

twice daily. The bulb of the thermometer was placed on a level with the terminal bud of the tubers. The variation among the different pots was always less than a degree. The average temperature one inch below the surface of the soil in pot 1, table XIV, was 18°5 C. for the 135 days.

The elimination of the rest period by removing the skin is not due to water absorption from the exterior, as tubers with the skins removed will sprout even in dry storage much earlier than those with skins intact; see table XVII.

TABLE XVII

EFFECT OF REMOVING THE SKINS AND STORING IN A DRY PLACE; MATURE TUBERS HARVESTED ON NOVEMBER 4; SKINS REMOVED ON NOVEMBER 7 AND PLACED IN MOIST CHAMBER 4 DAYS, THEN IN PAPER SACKS; STORED IN LABORATORY CUPBOARD

SKINS	PERCENTAGE SPROUTED AFTER		
	45 days	64 days	85 days
On	0	30	100
Removed	80	100	100

EFFECT OF CUTTING THE TUBERS IN HALF.—All the experiments so far reported were conducted with whole tubers, in which case sprouting always began first from the eyes on the seed end. The reverse is true, however, when the tubers are cut in half transversely to the long axis, separating the seed from the stem end. This applies only to tubers forced to sprout during the natural rest period. At the end of the rest period there seems to be little difference, in respect to time of sprouting, between the eyes on the seed and stem ends if the tubers are cut in half.

On October 31 the skin was removed from tubers harvested on October 28. They were then cut in half. The halves were stood upright on wet soil and covered with wet excelsior. On November 15 all the stem halves bore sprouts from eyes located near the cut surface.

Two lots of 4 tubers each were selected from McCormick potatoes harvested on November 4. On November 8 the tubers were cut in 4 pieces in the manner shown in fig. 1. In addition the skins

FIG. 1.—Effect of cutting tubers in half; skins removed; stem ends at bottom, bud ends at top; tubers harvested November 4; experiment started November 8; photographed December 5.

were removed from the pieces in one lot; both lots were then placed on wet soil in pots and covered with excelsior, which was kept constantly wet. Within 10 days all the stem pieces with skins removed bore sprouts from eyes near the cut surface. The stem pieces with skins on began to sprout on the 20th day. Figs. 1 and 2 show the growth of sprouts on the 24th day. It will be seen from this experiment that even with the skin intact the buds near a cut surface begin to sprout much earlier than normally, provided the exposed surface is kept moist and suberization thereby retarded. The buds on the pieces with skins removed not only sprouted still earlier, but the sprouts also grew much faster.

On November 13, tubers harvested on November 4 were cut in half transversely to the long axis. The stem halves were then divided into two lots of 5 each. The cut surfaces of one lot were immediately dipped into warm paraffin. When the paraffin cooled, forming a thin layer over the surface, both lots were placed in paper sacks and stored in a dark, dry laboratory closet. On December 18 all of the paraffined halves bore sprouts from buds near the cut surface. The surface underneath the paraffin was still moist and the cell walls very little suberized. The paraffin in drying cracked from the edges sufficiently to allow free access of air. The cut surfaces of those not dipped in paraffin were dry and heavily corked; these did not begin to sprout until a month later. It seems very probable that the surfaces kept moist by paraffin and not allowed to suberize admitted something to the near-by buds which was not so freely admitted through the heavily corked surfaces. It could not be water, as the sprouting occurred in a dry atmosphere. The other alternative is oxygen. The earlier sprouting in the case of the paraffined pieces was not the result of heat applied to the cut surface by the warm paraffin; this was proved when the experiment was repeated, using a third lot which was dipped in paraffin, the paraffin being removed as soon as cold. This lot sprouted no earlier than the one not treated.

EFFECT OF LIGHT.—Planting tubers with the stem ends in soil and covering the seed ends with wet excelsior exposes the latter to subdued light; it is sufficient, however, to induce rather rapid greening of the exposed part of the tuber. It soon became evident by

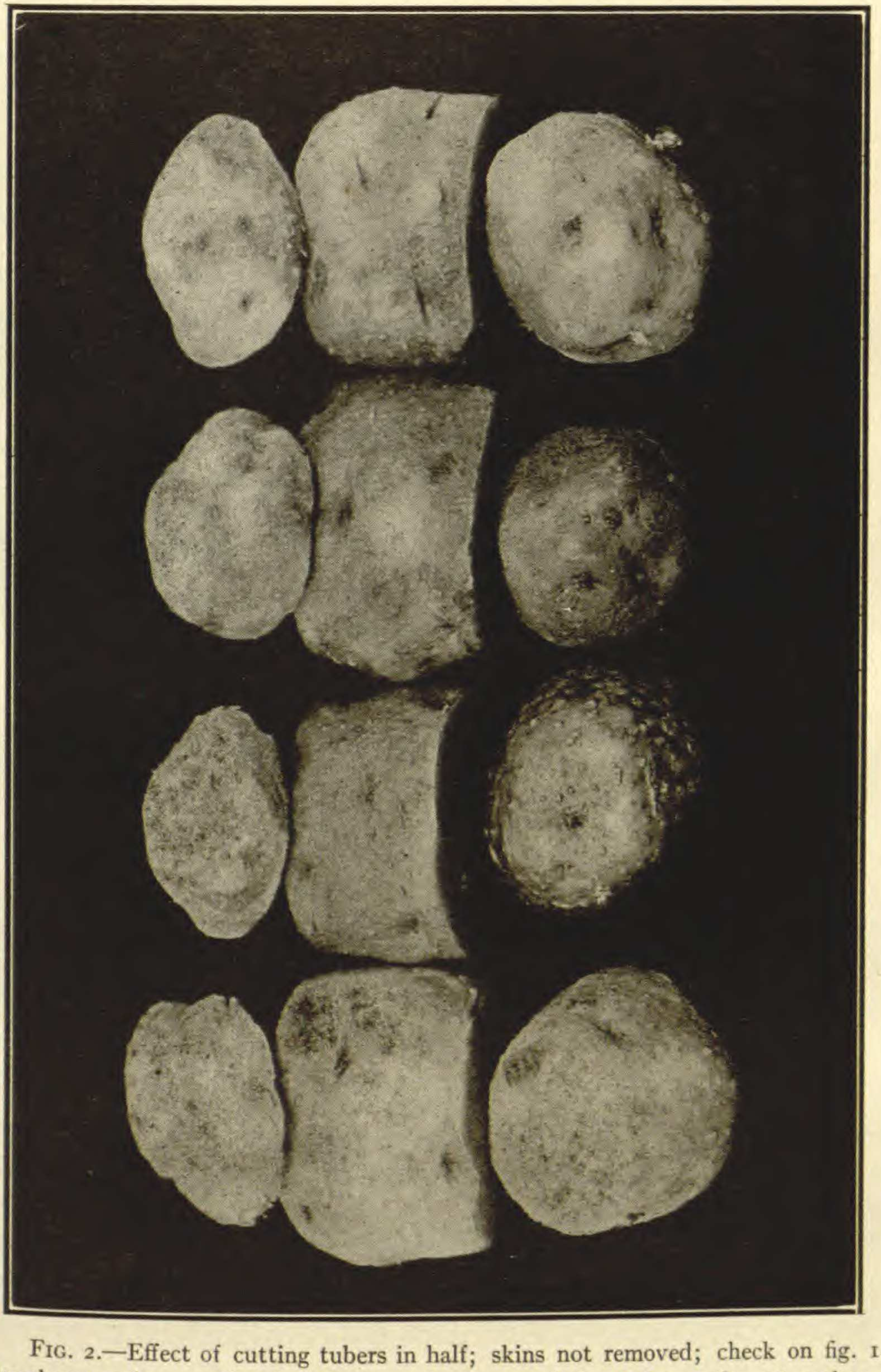

FIG. 2.—Effect of cutting tubers in half; skins not removed; check on fig. 1; also shows early sprouting from buds on the stem ends located near the cut surface.

the use of this method for sprouting tubers that light exercises an influence on the growth processes in the buds. Immature tubers with slightly suberized skins produce sprouts under the influence of subdued light and moisture almost as quickly as they do with the skins removed; see tables XIV and XVIII.

TABLE XVIII

EFFECT OF SUBDUED LIGHT ON IMMATURE TUBERS WITH SLIGHTLY SUBERIZED SKINS; 8 TUBERS IN EACH LOT

SEED HALVES COVERED WITH	PERCENTAGE SPROUTED AFTER			
	20 days	30 days	40 days	50 days
Excelsior	43	43	51	100
Excelsior and black cloth	0	0	14	43

The foregoing light effect is entirely balanced when the skins are removed; for sprouting occurs just as early in the dark as in subdued light, other conditions being comparable (table XIX).

TABLE XIX

EFFECT OF LIGHT WHEN THE SKINS ARE REMOVED; 12 TUBERS IN EACH LOT HARVESTED ON OCTOBER 28 AND PLANTED ON OCTOBER 31

STEM ENDS PLANTED IN	SEED ENDS COVERED WITH	PERCENTAGE SPROUTED AFTER		
		10 days	20 days	35 days
Soil	Excelsior and black cloth	25	83	83
Soil	Excelsior	25	83	100

Four lots of 5 tubers each were chosen from immature McCormick potatoes harvested on September 20 and immediately planted in pots with the stem ends in the soil. The projecting seed ends of the 4 lots were covered as follows: (1) a double-walled bell glass filled with a solution of ammoniacal copper sulphate; (2) a similar bell glass filled with a nearly saturated solution of potassium dichromate; (3) a clear bell glass; (4) a black-walled bell glass. By means of bent tubes the air under the bells was in free communication with that on the outside. Through these tubes water was added daily to each pot in sufficient amounts to maintain under the bells a nearly saturated atmosphere. Equal quantities were added to each pot. Thermometers were placed inside the bell glasses and the temperatures recorded early in the morning and in the afternoon. The temperatures ran practically the same under all the bells, except the clear one, which showed an average of 1–2° higher than the others (table XX).

TABLE XX

EFFECT OF LIGHT ON SPROUTING; IMMATURE TUBERS WITH SLIGHTLY SUBERIZED SKINS

STEM ENDS IN	SEED ENDS COVERED WITH	PERCENTAGE SPROUTED AFTER			AVERAGE LENGTH OF SPROUTS AFTER 35 DAYS
		10 days	20 days	35 days	
Soil..........	Clear bell	100	100	100	8 mm.
Soil..........	Red bell	20	100	100	5
Soil..........	Blue bell	0	0	20	1
Soil..........	Black bell	0	0	0	0

The experiment recorded in table XX shows not only the stimulating effect of light on the growth processes in the buds, but also suggests that this effect is due to a greater oxygenation of the tissues by photosynthesis. The chief evidence for this conclusion lies in the fact that the tubers under the blue bell glass sprouted very little earlier than those under the black bell glass. Although the tubers under the blue bell glass soon became green, little photosynthesis would be expected, on account of the slight energy for this process in the actinic rays. Subdued light does not hasten the sprouting of the tubers with well suberized skins; the effect is rather one of slight retardation. The rest period of tubers with heavily suberized skins may be considerably extended by thorough greening in full light.

EFFECT OF HYDROGEN PEROXIDE.—If the skin is in any degree permeable to hydrogen peroxide, the abundance of catalase in potato tubers would decompose it, liberating free oxygen. Earlier sprouting would then be expected if oxygen is a limiting factor for growth under normal conditions. Experiments to test this hypothesis were conducted as follows: Tubers were wrapped in cotton saturated with dioxygen, then stored in moist chambers, which were buried in wet sawdust underneath a greenhouse bench.

Untreated tubers were planted in the sawdust just outside of the moist chambers. The sawdust was kept constantly wet (table XXI).

TABLE XXI

EFFECT OF WRAPPING TUBERS IN COTTON SATURATED WITH HYDROGEN PEROXIDE; 6 TUBERS IN EACH LOT

Cotton saturated with	100 per cent sprouted after
3 per cent dioxygen.	28 days
100 per cent dioxygen.	43
Untreated.	76

Table XXI is typical of a number of experiments that were performed with new potatoes. Such treatment failed, however, to shorten the rest period of tubers with heavily suberized skins; this alteration of the skin doubtless renders it impermeable to hydrogen peroxide. Numerous attempts to control these experiments by wrapping tubers in cotton saturated with distilled water usually failed on account of the decay of the tubers. It may be assumed, however, that the partial oxygen pressure in the wet sawdust would not be less than that under saturated cotton.

RESPIRATION.—It has been shown that the rest period of potato tubers can be either entirely eliminated or greatly shortened by means which would seem to facilitate the oxygenation of the internal tissues. That a great increase in oxygen absorption actually occurs is proved conclusively by the effect of the various treatments on respiration, the rate of which was determined by the amount of carbon dioxide expired from the tubers. Ten tubers, with a total weight of about 1500 gm., were used for each determination, which was allowed to run 24 hours at room temperature. The control determinations were made at the same time and under exactly the same conditions.

The amount of carbon dioxide expired from new potatoes with thin, slightly suberized skins is much greater than that from the same tubers after the skins have become well corked and adherent to the underlying tissues. When the latter character of the skin is attained, the rate of respiration under uniform conditions remains fairly constant until the beginning of sprouting. Table XXII is a typical experiment which will suffice to show this fact. Immature

Rehobeth potatoes grown in the greenhouse and harvested on January 21 furnished the material for this experiment. The tubers were stored in a dry, warm laboratory drawer.

TABLE XXII

RELATION OF RESPIRATION TO SKIN SUBERIZATION

Date of determination	Thermograph average of room temperature	Milligrams of CO_2 per kilo per hour
January 21	20°3 C.	24.7
January 26	20.0	22.4
February 2	21.1	12.3
February 26	22.2	8.26
March 3	22.0	8.08

Removal of the skin from mature tubers more than doubles the rate of respiration; it falls again with the formation of a new thoroughly corked skin to that of unpared tubers (table XXIII).

TABLE XXIII

EFFECT OF REMOVING THE SKIN

DATE OF DETERMINATION	MILLIGRAMS OF CO_2 PER KILO PER HOUR		RATIO
	Untreated	Skins removed	
November 7	14.94	34.7	1:2.32
November 10	12.02	26.37	1:2.19
December 2	10.9	11.8	1:1.08

McCALLUM (**17a**) found ethyl bromide especially effective in forcing the resting buds of potatoes into growth. The writer studied the effect of this treatment on respiration in the tuber and found that it has about the same accelerating effect as removing the skin (table XXIV).

It has been shown that the rest period can be shortened by wrapping the new tuber in cotton saturated with hydrogen peroxide. This treatment also accelerates the rate of respiration in new tubers (table XXV).

It is obvious that the elimination or abbreviation of the rest period under the conditions employed in this work is correlated with greater oxygen absorption. It does not necessarily follow, however, that sprouting was brought about by the greater energy release resulting from the increased respiration. The facts seem to indicate that this was not the case. It is more probable that under normal conditions the skin becomes suberized before the completion of some growth mechanism requiring oxygen. The rate of oxygen diffusion through the suberized skin then determines the time, the natural rest period, required for the perfection of the growth mechanism.

TABLE XXIV

EFFECT OF TREATMENT WITH ETHYL BROMIDE GAS

DATE OF DETERMINATION	MILLIGRAMS OF CO_2 PER KILO PER HOUR		RATIO
	Untreated	Ethyl bromide gas—30 minutes	
January 18..........	7.26	28.54	1:3.93
January 21..........	7.08	27.85	1:3.83

TABLE XXV

EFFECT OF TEATMENT WITH HYDROGEN PEROXIDE; NEW TUBERS

EXPERIMENT	MILLIGRAMS OF CO_2 PER KILO PER HOUR	
	Lot 1	Lot 2
1............	Untreated..........22.41	Untreated..........24.65
2............	Wrapped in sterilized cotton saturated with sterilized water........22.9	Wrapped in cotton saturated with 50 per cent dioxygen.............. 39.3

The rôle of oxygen in plant physiological processes is very complex and at the present time quite obscure. However, several cases are noted in the literature which show the wide range of oxygen influence in growth processes. IVANOFF (11) claims that oxygen is necessary for the transformation of zymogen into zymase. ZALESKI (32) has shown that protein synthesis is influenced by oxygen, etc.

Summary and conclusions

Under normal planting conditions potato tubers will not sprout for several weeks after harvest. During this rest period certain changes must occur in the chemical or physical situation of the buds or their immediate environment which are essential to the release of the growth processes. These changes are spoken of as "after-ripening," using the term in its broadest sense.

The carbohydrate transformations during the rest period are dependent entirely upon changing temperature.

Active diastase and invertase are present at all stages of the rest period, but show no increase under normal growing conditions until the tubers begin to sprout.

The juice from tubers at the end of the rest period causes a greater acceleration of the oxidation of pyrogallol than the juice from new immature tubers of the same variety. The seed and stem halves show no difference in the ability to oxidize pyrogallol even after sprouting from the seed end.

After-ripening does not involve protein hydrolysis. There is no change during the rest period in the relative magnitudes of the following forms of nitrogen: proteose and peptone; diamino acids and other bases; monoamino acids and amides.

Protein, lipoid, organic extractive, and inorganic phosphorus calculated to percentage of total phosphorus, each remains constant up to the time of sprouting.

The magnitudes of the substance studied are not all identical in the seed and stem halves. The relative composition, however, remains practically constant during the rest period in spite of the fact that sprouting begins much earlier on the seed end.

Metabolic changes involving the above forms of nitrogen and phosphorus begin rather suddenly and are concurrent with sprouting. The same is true of diastase.

Drying causes rapid suberization of new skin and exposed surfaces.

Suberization greatly reduces the permeability of the skin to water and gases.

Potatoes may be sprouted at any time during the rest period by simply removing the skins and supplying the tubers with favorable growing conditions, which include in this case the maximum partial oxygen pressure of the atmosphere. The elimination of the rest period by this means is not due to water absorption from the exterior, as tubers with skins removed will sprout, even in dry storage, much earlier than those with skins intact.

If tubers are cut in half transversely or cut into half-inch slices, the buds on the stem half located near the exposed surface will sprout much earlier than normally, provided suberization of the surface cells is prevented. This may be accomplished by laying them on wet soil, or, better still, sawdust, and covering with wet excelsior. Sprouting in this case also was not due to water absorption, because the rest period of these buds may be greatly shortened in dry storage if drying of the exposed surface is prevented by covering it with a thin layer of paraffin.

The earliest sprouting occurred when the skins were removed and the tubers also cut in the manner described above.

Subdued light stimulates growth in buds on new tubers with slightly suberized skins. The evidence at hand makes it highly probable that this effect is due to greater oxygenation of the tissues by photosynthesis. The light influence entirely disappears when the skin is removed. Subdued light does not stimulate growth in the buds on tubers with highly suberized skins; the effect is rather one of retardation.

The rest period of new potatoes may be shortened by wrapping the tubers in cotton saturated with hydrogen peroxide. The abundance of catalase in potato tuber decomposes the hydrogen peroxide diffusing through the thin skin, liberating free oxygen. This treatment had no effect on old tubers on account of the impermeability of the heavily suberized skin to the hydrogen peroxide.

All the foregoing treatments greatly accelerate the rate of respiration. It may be safely concluded, therefore, that the elimination or abbreviation of the rest period under the conditions employed in this work is correlated with increased oxygen absorption.

The rest period of the potato tubers is not firmly fixed and hereditary. It is not of internal origin due to autogenic metabolic changes, as it can be entirely eliminated by means which maintain a proper adjustment between the bud tissues and external agents, chiefly oxygen. In nature the oxygen supply to the internal tissues is regulated by skin characters, which are greatly influenced by moisture regulations.

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